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Can Tissue Anomalies that Occur in Marine Fish Implicate Specific Pollutant Chemicals

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16. ABSTRACT

The advantage of using tissue abnormalities in wild fish as a measure of fish health is that the abnormality, unlike sensitive biochemical anomalies, cannot be said to have occurred during caputre or transport. This paper is concerned with factors (chemical, physical, or biological) in polluted waters that cause pathology.

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Can Tissue Anomalies that Occur in Marine Fish Implicate Specific Pollutant Chemicals?

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INTRODUCTION

The advantage of using tissue abnormalities in wild fish as a measure of fish health is that the abnormality, unlike sensitive biochemical anomalies, cannot be said to have occurred during capture or transport to the lab. It usually takes hours, days, weeks and sometimes even months for abnormal tissue pathologies to develop. The researcher can be confident that some factor in the field caused the abnormality. When an abnormal fish is captured, logical questions appear at four different levels: (1) What is the structure or morphology of the abnormality? Many scholarly articles by histopathologists describe in detail tissue anomalies observed in field specimens. At this level the focus is on the pathology itself. (2) What is the incidence of the pathology in the population? How many are afflicted, old or young, male or female? Here the focus is on the species population. (3) Can the incidence of a fish disease be linked to environmental pollution? Here the focus is on correlating pathology with pollution. (4) What factor or factors (chemical, physical and/or biological) in the polluted waters caused the pathology? This is probably the most difficult question to answer and is the subject of this paper. Answers to the first three questions must be found before attempts can be made to answer the fourth. If a specific fish disease can be linked to a specific xenobi-

otic, then another tier of questions arises: (5) What is the significance of this to human health and well being? Is a food source diminished? Are humans ingesting fish containing toxic chemicals? What is the aesthetic/economic cost versus the industrial/economic gain of having continued pollution? Answers to these questions are beyond the scope of this paper, which will focus on the question of whether there are pollutant-specific pathologies in marine fish.

CAUSES OF TISSUE ABNORMALITIES IN FISH

From the results of voluminous laboratory studies it is known that many chemicals and other agents can cause abnormalities in fish. But what are the natural causes of anomalies in the field that will complicate designation of a lesion as anthropogenic? Some of the most significant causes of disease in marine fish are biological agents such as viruses, bacteria, fungi, protozoa, helminthes and parasitic copepods (Sindermann 1966, 1979; Love et al. 1981). Many of these infections result in tumors and lesions that persist in the living fish. Fish are also subject to epizootics or plagues in which large percentages of a population are simultaneously killed or infected by a single infectious pathogen. Outbreaks of the fungal pathogen, Ichthyophonus hoferi, in herring of the western Atlantic have been known since 1898, and Sindermann (1966) reports that in 1954-55 an estimated one-half of the entire herring population of the Gulf of St. Lawrence was killed by the disease.

Another disease with a long history of epizootic prevalence is the "red disease" of eels caused by Vibrio anguillarum. Italian literature dating back to 1718 records repeated and severe outbreaks on the coast of Italy throughout the eighteenth and nineteenth centuries (Sindermann 1966). There is evidence that epizootics occur when fish populations reach high densities and when summer temperatures allow the rapid proliferation of bacteria (Sindermann 1966). The possibility that fish pathogens are also human pathogens cannot be ruled out. For example, Love et al. (1981) isolated a Vibrio from naturally occurring skin ulcers on a damselfish and from human wounds.

Inherited abnormalities and inherited propensities for disease undoubtedly also exist in the field. Unfortunately data in this area are scant. Gordon (1954) reviewed the genetics of fish diseases.

Another cause of deformed fish in the field is starvation. O'Connell (1980) found irregular shaped anchovy larvae off the coast of San Diego, Calif., and concluded that their emaciated condition was very similar to that induced in laboratory animals by total food deprivation. A condition known as "jellied" flesh that occurs in several flatfish species in cold water (Templeman and Andrews 1956) may also be caused by starvation. Other causes of anomalies in wild fish can be grouped under the heading of physical

environmental factors such as salinity, temperature, pH or O_2 concentration. Table 1 summarizes the categories of disease-causing factors in wild fish. It must be emphasized that multiple factors can be involved in any disease occurrence. For example, warm water may induce a microbial infection of a genetically predisposed population. A toxic chemical may cause immunosuppression in a population which then contracts a viral disease. A starving population may be killed by low oxygen when a well-fed population would survive.

Table 1. Causes of anomalies in wild fish.

1. Nutritional (starvation, etc.)
2. Biological (bacteria, viruses, fungi, protozoans, helminthes, etc.)
3. Genetic
4. Physical environmental (pH, temperature, salinity, O_2, etc.)
5. Chemical (red tide, xenobiotics, petroleum, etc.)

VARIATION IN DISEASE PREVALENCE IN MARINE FISH

The prevalence of anomalies in fish populations may vary with geography, time, age, sex and diet. Figure 1 shows the geographic incidence of two diseases of flatfish. Skin papillomas, a disfiguring protozoan disease of flatfish, occurs in the Pacific but not in the Atlantic. By contrast, the well-studied viral disease lymphocystis occurs primarily in the North Atlantic flatfish with no recordings in the Pacific south of Alaska. Superimposed on the global distribution pattern, however, are local variations in tumor presence from 58 to 0.01% of a population (Stich et al. 1977). Temporal variation in prevalence of disease is best illustrated by studies of epizootics. Sindermann (1966) lists a sequence of events in the Ichthyophonus disease of herring which includes a long enzootic phase of very low prevalence (< 0.1%), several years of rapidly increasing incidence which reaches an epizootic peak when about 25% of the individuals are infected and acute infections are common, then several years of rapidly declining incidence and return to the enzootic phase.

Disease in Pristine Alaskan Waters

An excellent example of a comprehensive survey of disease prevalence in wild fish is that of McCain et al. (1979) in Alaskan waters. A review of this study will further illustrate some of the kinds of variance that exist in disease prevalence. Presumably the Bering Sea stations used in this study (Figure 2) were relatively

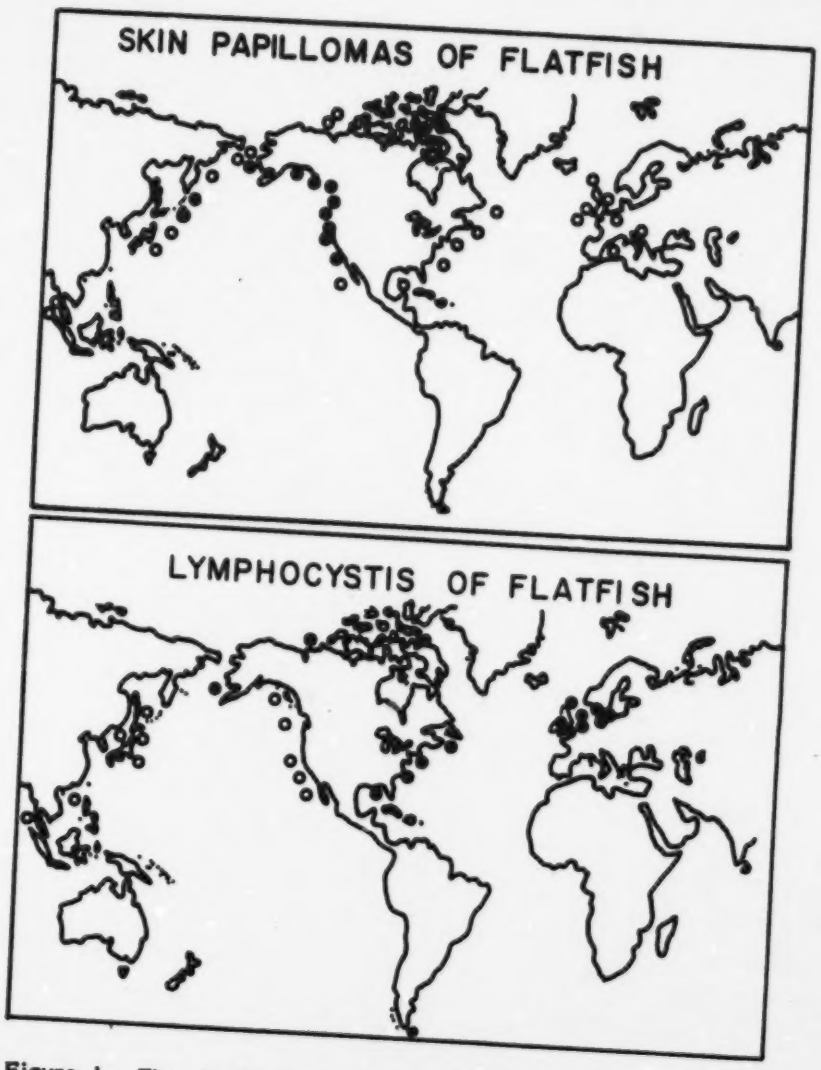

Figure 1. The distribution of two microbial diseases of flatfish. Black dots show sample stations where the disease was found. Open circles show stations where disease was absent. (After Stich et al. 1977).

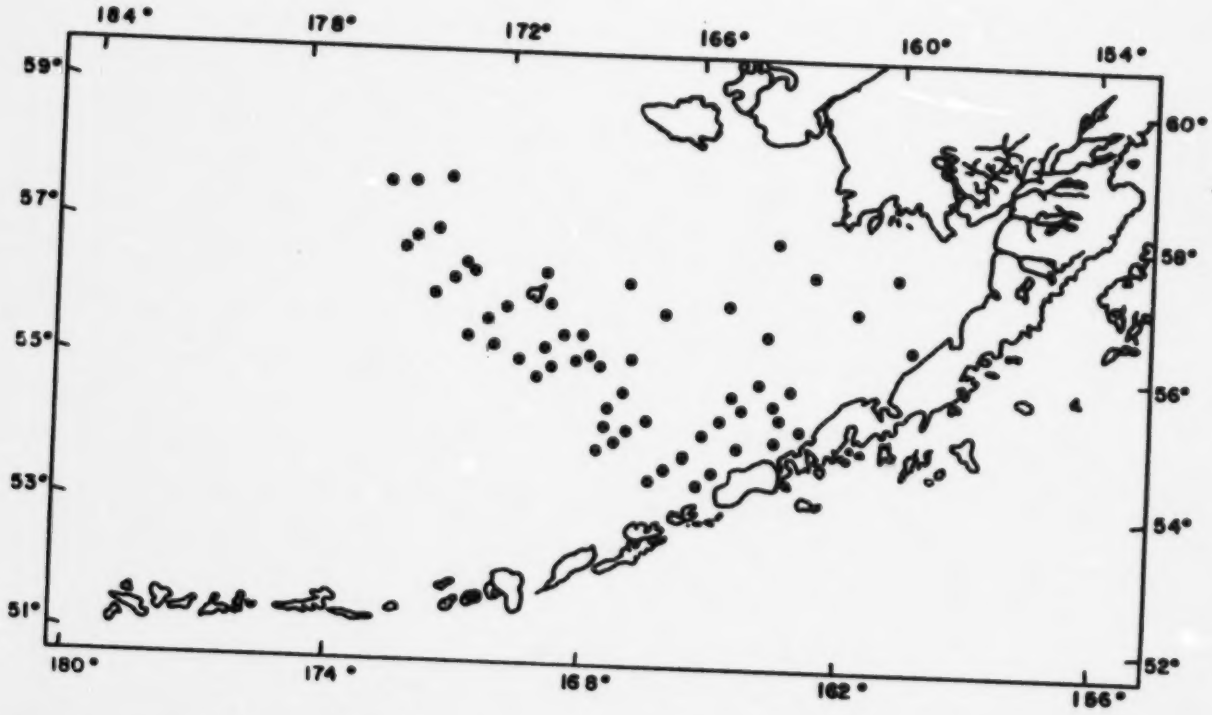

Figure 2. Location of sample sites (black dots) that were used in the McCain study of diseases of marine fishes in Alaskan waters. (After McCain et al. 1979).

pristine. A total of 36,618 fish were captured and examined for pathological conditions. Of the 26 species examined, only four had detectable abnormalities. Among Pacific cod, 8.7% had pseudobranchial tumors, and 1.6% had skin ulcers. Among walleye pollock, 1.7% had pseudobranchial tumors. Of yellowfin sole, 2.8% had lymphocystis and 1.4% of rock sole had epidermal papillomas. Pseudobranchial tumors, like the papillomas, are caused by protozoans (Dawe 1979). The tumors of the cod and pollock were distributed throughout the sampling area. The highest frequencies of sole with lymphocystis were in the southeastern Bering Sea. Figure 3 shows a composite of frequency-length profiles for four of the species along with lesion incidence. Note that a higher percentage of the small pollock (young) had tumors than the large. Also note that young male rock sole had a higher frequency of papillomas than young female sole. McCain et al. (1979) suggested that the occurrence of papillomas was depth related since the young individuals were captured in shallow water and the old in deeper water. This study illustrates the importance of patchiness, sex and age differences and species specificity and the need for large numbers of fish and sample sites for understanding variance in disease prevalence.

Deformities Near Los Angeles

Valentine's (1975) survey of gill raker deformities in barred sand bass from Southern Calif. presents data on a field anomaly that may have been associated with pollution. Here there were two sample areas. One area included six stations located between Los Angeles and San Diego; the other was 500-700 miles south of San Diego in Baja, California. The incidence of severely deformed gill rakers in Southern California was 30.5%; the incidence in Baja was 0%. Figure 4 shows the relationship between size and incidence of the deformity in California fish. Valentine asserts that sand bass are not migratory and that two additional species, the California grunion and the barred surf perch, also exhibited gill raker abnormalities when captured in California waters. Unfortunately we do not know whether the grunion and surf perch from Baja had lesions. During the time that this study was conducted (1967-1971), hundreds of kilograms of PCBs and DDT were being discharged into Los Angeles coastal waters (Schmidt et al. 1971) along with tens of thousands of kilograms of phosphates, nitrates, coliforms, metals and other substances. Valentine speculated that the abnormalities were either of genetic origin or caused by pollution and cited references showing that heavy metals, DDT and PCBs all can interfere with calcium metabolism: here was a study that comes as close as most to implicating pollution. Unfortunately, the concentrations of pollutants in the sample areas were not fully known. The concentrations of toxicants in the fish were not determined and the distribution of sample sites was too restricted.

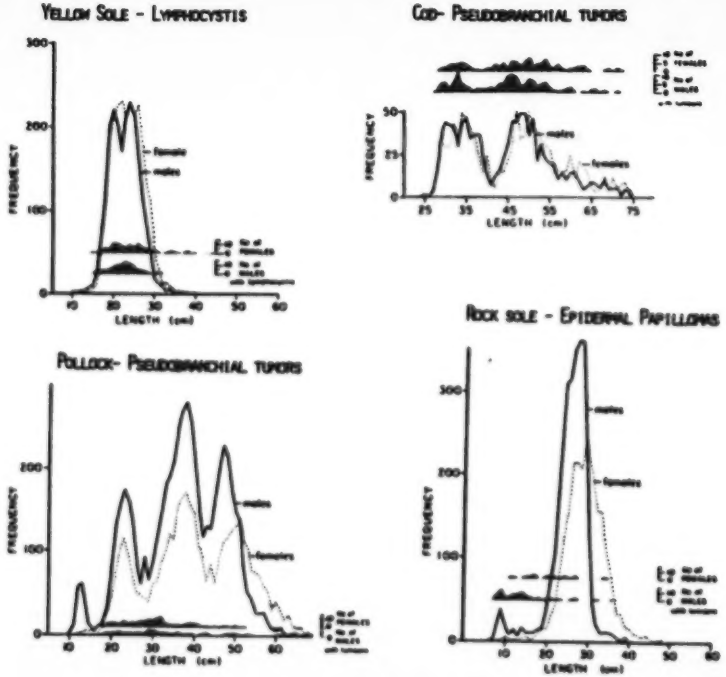

Figure 3. Frequency-length graphs for four of the species caught in the Alaskan study (in white). The incidence of a specific disease is shown in black. (From McCain et al. 1979, with permission of copyright holders, Blackwell Scientific Publications Limited)

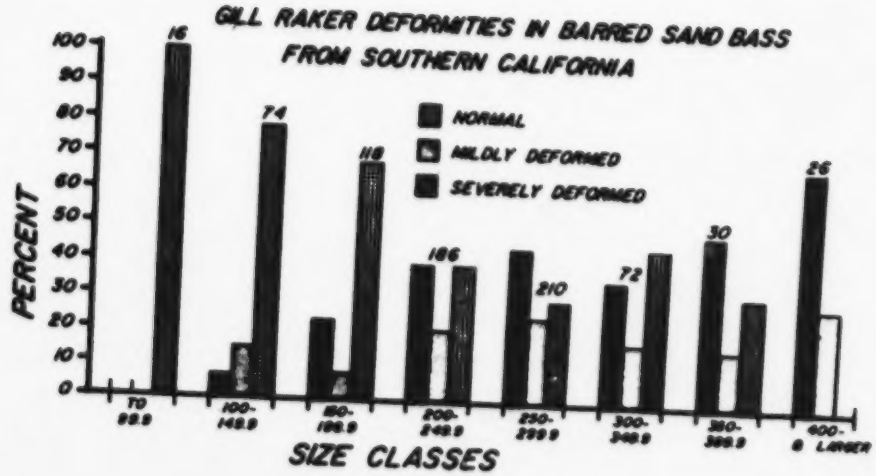

Figure 4. Bar graph of the incidence of normal, mildly deformed, and severely deformed gill rakers in barred sand bass from southern California. With increasing size (age), expressed in standard length (mm), the percentage of normal gill rakers decreases while the percentage of deformed gill rakers increases. All specimens represented in this figure were captured between 1967 and 1971, inclusive. The percentages of severely and mildly deformed and normal gill rakers respectively are as follows (size classes followed by three percentages): To 99.9 mm, 0.0, 0.0, 100.0; 100-149.9 mm, 6.8, 14.9, 78.3; 150-199.9 mm, 22.9, 8.5, 68.6; 200-249.9 mm, 39.2, 21.0, 39.8; 250-299.9 mm, 45.2, 24.8, 30.0; 300-349.9 mm, 36.1, 18.0, 46.0; 350-399.9 mm, 50.0, 16.7, 32.3; 400 mm and larger, 69.2, 30.8, 0.0. (From Valentine 1975 with permission of copyright holders University of Wisconsin Press and the Board of Regents of the University of Wisconsin System.)

There are many other examples in the literature that indirectly implicate anthropogenically contaminated environments with anomalies in feral fish, without direct proof of the association.

Liver Cancer in the Duwamish River

An example of a single species survey in which pollution effects are suggested but not proved is that of Pierce et al. (1978) using data for fish in the Duwamish River Estuary, Seattle, Wash. Figure 5 shows the four stations used: West Point and Alki Point are both sewage outfalls and the Duwamish River is a highly polluted ship channel. Point Bully was the single control site. Figure 6 summarizes the results of the analyses of English sole livers collected from the four sites. Eleven types of liver lesion were distinguished; for example, type 1 was fatty liver, type 11 was hepatoma. Clearly, most of the flatfish in the Duwamish River possessed diseased livers. Fish from both Alki and West Points also exhibited high incidence of lesions (although no cancer). Even fish from the control area (Point Bully) exhibited lesions.

Duwamish River sole contained 1-5 ppm PCB as a body burden. The authors cited laboratory studies showing PCBs caused lesions in fish similar to those seen in the sole, with the exception of hepatomas and concluded that "although environmental chemical contaminants were suspected as being the etiologic agents for the liver lesions, the establishment of cause must await additional investigations. . . ." Although inclusion of more control sites with documentably distinct populations would have strengthened the study, a correlation between pollution and fish tissue anomalies does appear to exist. Taking the next step (answering question #4) and determining the precise cause of liver lesions presents a challenge to the researchers. Like most cities, Seattle waters are polluted by a highly complex mixture of chemicals which interact with each other, the environment and biota in numerous ways.

Fin Erosion or Fin Rot

This abnormality is the best example of a disease of fish inhabiting polluted waters. Sindermann's (1979) review covers the relevant literature. An association between occurrences of fin rot and high sediment coliform counts (Mahoney et al. 1973) and high concentrations of heavy metal in sediments (Caromody et al. 1973) has been reported. The signs of disease can be produced experimentally by exposure of fish to polluted sediments, by laboratory exposure to 3-5 $\mu g\ L^{-1}$ of Aroclor 1254 (Couch and Nimmo 1975), or by exposure to 4-5 ppm crude oil (Minchew and Yarbrough 1977). Fin erosion has been observed in striped bass overwintering in heated effluents of power plants in the Middle Atlantic states (Sindermann 1979). At least 50 species of fish have been captured with fin rot. Many, on the other hand, appear to be immune to the disease. For example, the English sole with the very high levels of liver abnor-

Figure 5. Locations of the four sample stations used in the Duwamish River study. (After Pierce et al. 1978)

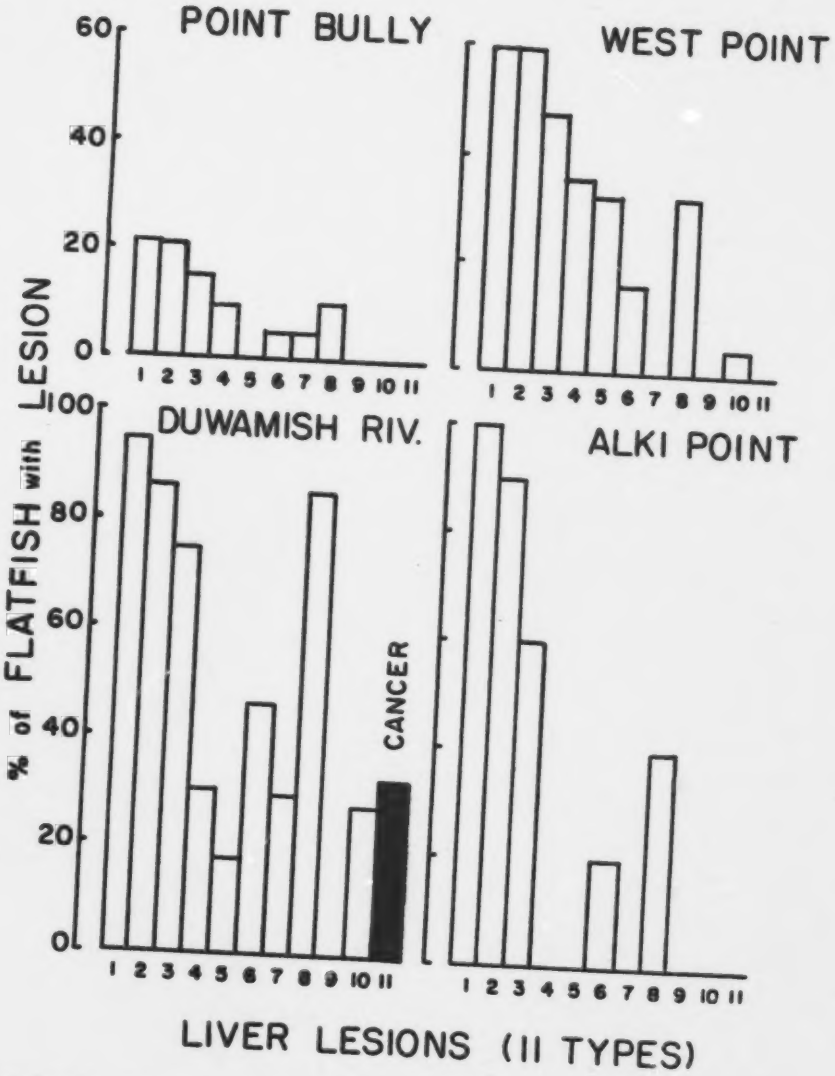

Figure 6. Percentages of English sole with different types of liver lesions from the four sample stations in Figure 5. Each number from 1 to 11 represents a different liver lesion (e.g., 1 is fatty liver, 11 is liver cancer). (Redrawn from Pierce et al. 1978)

malities in the polluted Duwamish River (Pierce et al. 1978) had only a 0.5% incidence of fin erosion while as many as 60% of all stargazers (Uranoscopus japonicus) from Suruga Bay, Japan, had eroded fins (Nakai et al. 1973). Bascom (1982) rejects bacteria, abrasion, macroparasites, fin nipping, H_2S in interstitial water and high metal concentrations as causes of fin erosion and suggests a principal chemical cause. This favoring of a principal chemical cause is also put forth by Sindermann (1979). And Wellings et al. (1976) also suggest "that the incidence of fin erosion in a particular population is related to an interaction between genetic constitution and multiple environmental variables, including a variety of chemical pollutants." Thus, the specific cause of the best known pollutant-associated fish disease remains unknown.

Hypothetical Correlations of Lesions and Pollution

Before specific chemicals can be implicated as causative agents of fish lesions (question 4), a positive correlation between incidence of a lesion and incidence of anthropogenic pollution must be established. Figure 7 illustrates four potential hypothetical relationships that could exist between lesion occurrence in a fish population and a site of pollution. There are, of course, many ways to plot the data. The curves presented do not allow for patchiness and water born pollution probably would not emanate from a city in a symmetrical fashion. Nevertheless, Figure 7 demonstrates that pollution can have opposite effects on disease incidence. In case A, disease in a given species exhibits no change in incidence among individuals in the polluted region; a possible example could be the case of the anisakine roundworm infection of fishes off California, where visceral tissue of 41.6% of the fish examined (2268 fish, 68 species) contained worms (Dailey et al. 1980). In case B the incidence of disease decreases among individuals in a polluted area; this could be interpreted as the additional stress of the degraded environment killing off many of the diseased animals. In case C, the incidence of disease increases in a polluted area, where the immune system of normally uninfected individuals may be repressed in the polluted area so that a larger percentage of individuals contract the disease. Finally, in case D, the disease is found only in fish inhabiting a polluted area, with fin erosion apparently one of the few known examples. Relationships among most of the diseases of wild fish and environmental contamination with chemicals are largely unknown. To establish such a correlation, the pollution source must be well within the range of fish sampling sites and must include appropriate control sites. Furthermore, fish must not migrate among the sites. In addition, the fish sampling sites should serve as chemical sampling sites, where levels in tissue, sediment and water of indicator pollutants are measured. Ideally, pathology and chemistry are done in the same laboratory.

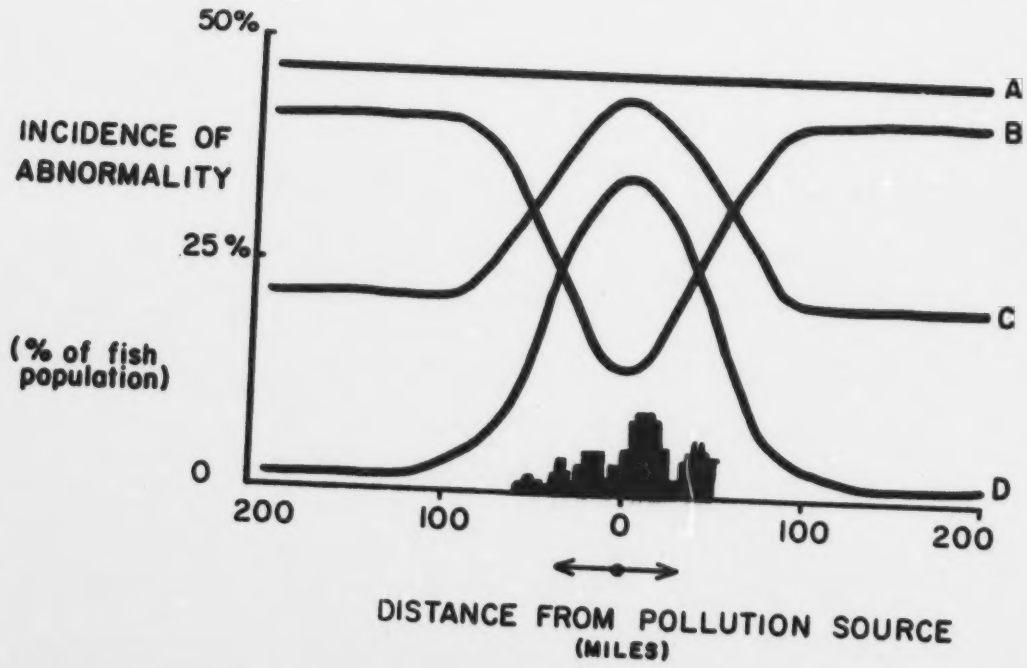

Figure 7. Four hypothetical relationships between disease incidence in marine fish and a site of chronic anthropogenic pollution. (A) No change in disease incidence with pollution. (B) Negative correlation—disease incidence decreases near pollution. (C) Positive correlation—widespread disease increases in incidence near pollution. (D) Positive correlation—disease and pollution occur together.

PATHOLOGIES INDUCED IN FISH BY CHEMICALS IN THE LABORATORY

Interspecies differences in the metabolism of xenobiotics can be dramatic. Figure 8 summarizes results of a PCB bioaccumulation study in which the same five PCB mixtures were fed to different vertebrates and each species accumulated a different amount of isomers (Sparling and Safe 1980).

Some species show no reaction to doses of toxicants that are lethal for other species. When a chemical causes a lesion in a species, the question to be asked is whether that lesion is specific to the chemical. There have been many laboratory studies of chemically-induced histopathological changes in fish. The impact of petroleum exposure on fish has received attention, and such studies illustrate the difficulty in gaining an understanding of pollution effects when a polluting material contains thousands of different chemicals (Malins and Hodgins 1981; Malins 1982). Often the relevance of these studies to the field are questionable because the doses of toxicants employed were higher than most feral fish ever encounter. Single chemicals are often used when, in the environment, pollution is usually a multi-chemical bombardment. Also, fish avoidance behavior can be blocked by confinement. Whether relevant in the field or not, laboratory exposure studies have clearly shown that many chemical agents may cause similar tissue pathologies. This apparent difficulty in using tissue responses to specific chemicals may be overcome, perhaps by relying on syndromes of responses and not on single, limited effects. Responses obtained in multiple end point studies can then lead to a holistic linking of cause and effect, in certain cases (Couch et al. 1977).

Fatty Liver

Known causes of triglyceride accumulation in liver (fatty liver) are many and these include starvation, low protein diet, high fat diet, hormone imbalance, anoxic factors, bacterial toxins and chemical poisons (Popper and Shaffner 1957; Alpers and Isselbacher 1975). The best studied of the chemical poisons are carbon tetrachloride, chloroform, phosphorus and trinitrotoluene. Agents that cause fatty liver in captive fish include Denison tags (Saddler and Cardwell 1971), rancid fat (Smith 1979), hydrogenated fat (Cowey et al. 1976), 1 ppb PCB in the diet (Jensen et al. 1970) and crude oil in aquarium sediments (700 $\mu g\ g^{-1}$ dry sediment) (McCain et al. 1978). In an extensive review of the effects of pesticides on livers of fishes, Couch (1975) reported that the most commonly encountered liver lesion was abnormal fatty accumulation. Thus, fatty liver is a useful, nonspecific indicator of stress in fish, but it is a questionable indicator of the presence of a specific chemical. The ultrastructure of fish liver cells is nearly identical to that of mammals (Welsch and Storch 1973; Peute et al. 1978; Schoor and Couch

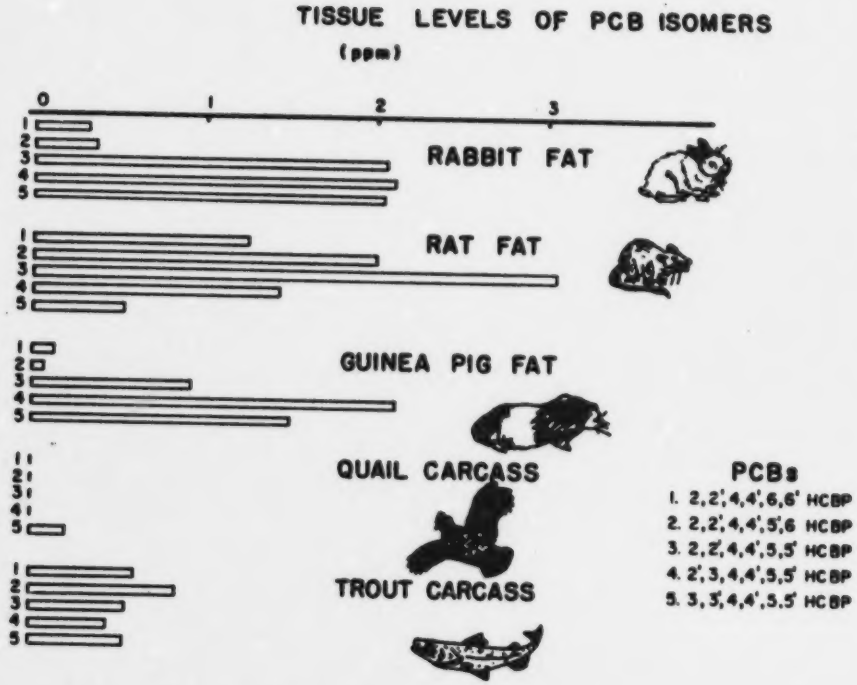

Figure 8. The concentration of PCB isomers in five animal species 29 days after a single feeding of an equimolar mixture of the isomers (5 mg kg^{-1}). Only fatty tissue was examined in the rat, guinea pig, and rabbit experiments. Whole carcasses were used in the trout and quail experiments. (From Sparling and Safe 1980)

1979). Some species, however, store reserve fat in their livers naturally (45%-67%) (Aure 1967). These species have lean flesh and are generally sluggish bottom dwellers, such as cod, snapper, haddock, perch and whiting. Other species store fat in muscle and possess lean livers identical to mammals (3%-5%), for example, salmon, mackerel, herring, shad, sturgeon, and tuna (Bilinski 1979; Welsch and Storch 1973; Bishop et al. 1976). Thus, what may be a pathological condition in one species can be normal in another. For a more complete discussion of hepatic toxicology in fish, see the excellent review by Gingerich (1982).

Gill Hyperplasia

Another nonspecific chemically induced lesion in fish is gill hyperplasia and hypertrophy which can lead to fusion of gill lamellae. Solangi and Overstreet (1982) found that two estuarine fish exposed to south Louisiana whole crude oil and/or its water soluble fractions developed gill epithelial hyperplasia. They noted, however, that similar lesions occur frequently in a variety of fishes exposed to various irritants such as heavy metals, pesticides, insecticides, ammonia and suspended sediments (Eller 1975; Smith and Piper 1975; Walsh and Ribelin 1975; Hodgins et al. 1977; and O'Connor et al. 1977).

Hepatoma (Liver Cancer)

Fish appear to be susceptible to all the types of cancers that humans get, and metastasis (spreading of the tumor to other tissues) appears to take place, although somewhat more slowly than in homeotherms (Lucke and Schlumberger 1949; Ashley 1969). Hepatoma formation has been induced in several teleost species by exposure to known hepatocarcinogens, including dimethyl-nitrosamine, acetylaminofluorene, urethan, thiourea, p-dimethylaminoazobenzene (Ashley 1969; Dollar et al. 1967; and Halver 1967). Hepatoma formation in hatchery-raised rainbow trout caused by contamination of their food supply with aflatoxin has been well documented (Yasutake and Rucker 1967; Ashley 1967; Dollar et al. 1967; Hendricks 1981). In addition to the study by Pierce et al. (1978) cited above, liver neoplasia has been found in feral hagfish (Falkmer et al. 1976, 1977) and feral white suckers (Dawe et al. 1964). Thus, like other nonspecific lesions cited above, i.e., fatty liver and gill hyperplasia, liver cancer occurs in wild fish and a variety of agents may cause it.

Inclusion Bodies

When metals and organics are bioaccumulated, they can be stored in accumulation or inclusion bodies that may be at least diagnostic of classes of toxicants if not specific chemicals. However, a harmful effect is not necessarily indicated. Lead, gold, iron, bismuth, uranium, beryllium, mercury, copper and arsenic are

a few of the metals that can be deposited intracellularly (Sorenson and Smith 1981). Intranuclear arsenic inclusions have been observed in hepatocytes of the green sunfish exposed to arsenic but not in channel catfish (Sorenson and Smith 1981). In the channel catfish 15 ppm arsenic exposure for 6 months caused a significant increase in the number and size of liver hemosiderin granules. Presumably these iron rich particles resulted from arsenic-induced erythrocyte lysis. Electron probe X-ray analysis data showed that about 30% of the hemosiderin granules contained small amounts of arsenic (Sorenson and Smith 1981). Thus, arsenic is related to the presence of different inclusion bodies (lesions) in different fish species and only in the green sunfish, Lepomis cyanellus, is the lesion an arsenic rich inclusion body.

Hawkes et al. (1980) examined the effects of petroleum hydrocarbons and chlorinated biphenyls on the morphology of Chinook salmon intestine. They found that after dietary exposure to petroleum hydrocarbons, the intestines contained inclusion bodies morphologically distinct from inclusion bodies that occurred when chlorinated biphenyls were fed to the fish. Unfortunately the composition of the inclusion bodies was not determined.

One of the best known of the chemically induced inclusion bodies in mammals is the "membrane whorl" or multilamellar body (Figure 9) that occurs in liver and other tissues. Recent electron micrographs of tissue from rats exposed to DDT and PCB show that these whorls are endocytotically expelled from the hepatocyte into the bile canaliculi (Jonsson et al. 1981). This is the most convincing evidence to date supporting the hypothesis that membrane whorls may be one vehicle whereby cells discharge specific hepatotoxic substances. More than 30 amphiphilic cationic drugs cause membrane whorls in mammals (Lüllman et al. 1978), and these lysosomally derived bodies appear to be the major site of toxicant accumulation (Matsuzawa and Hostetler 1980). Proliferation of rough endoplasmic reticulum occurs in fish liver following exposure to arsenic (Sorenson et al. 1980), petroleum (McCain et al. 1978) and benzo(a)pyrene (Schoor and Couch 1979). And proliferation of smooth endoplasmic reticulum occurs in Chinook salmon liver following dietary exposure to 5 ppm chlorobiphenyls for 28 days (Hawkes 1980). Characteristic ER whorls (myelin figures) also occur in hepatopancreatic cells of shrimp exposed to the PCB, aroclor 1254 (Couch and Nimmo 1974). Indeed, these findings of PCB effects on the ultrastructure of cells in an invertebrate (shrimp) anticipated the later findings in fishes cited above. Unfortunately membrane whorls have not been isolated from toxicant-exposed fish and compositions of the whorls determined. They appear, however, to be lesions suitable for further study since they may contain the causative chemical agent. It is not suggested, however, that these whorls necessarily constitute a harmful effect.

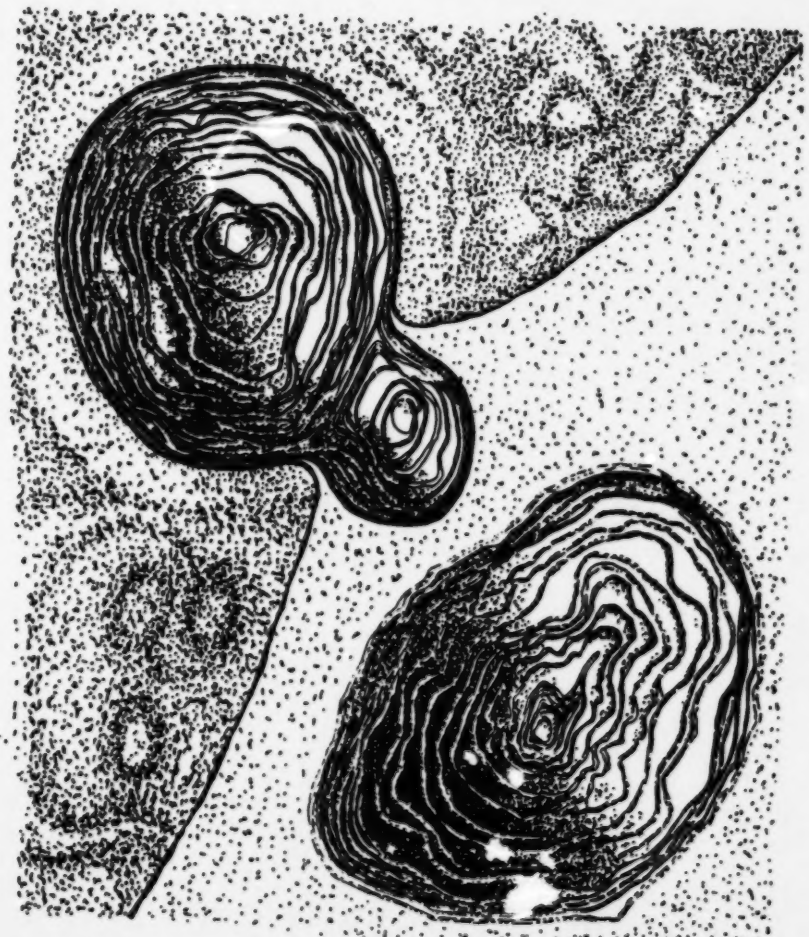

Figure 9. A liver membrane whorl (induced by DDT and PCB) that is undergoing exocytosis into the bile canaliculus. (Drawn from Jonsson et al. 1981)

From laboratory studies it can be concluded that many nonspecific lesions occur in organisms in response to environmental chemicals and few, if any, lesions have been proven to be toxicant specific. Isolation and chemical characterization of inclusion body lesions in wild fish may be the best means of determining causes of abnormalities in field specimens.

SCOLIOSIS, KEPONE AND THE JAMES RIVER FISH KILLS— COMBINED FIELD AND LABORATORY STUDIES

The following case study clearly illustrates the usefulness of experimental pathology in determining the probable cause of an abnormality in field specimens. In the spring of 1973 and 1974, massive kills of bluefish, spot and croaker entering the James River from the ocean occurred. R.J. Huggett (personal communication) observed that when sewage chlorinators draining into the river were turned off, the fish kills ceased. When they were turned on again, the fish kills occurred. Many of the species that were killed had broken backs (Figure 10C). It was then demonstrated in laboratory studies that chlorine could kill juvenile croaker, though no broken backs were observed in the chlorine-killed fish. Recently, Couch (1982) at the U.S. EPA Laboratory examined specimens of Cyprinodon variegatus exposed to chlorine (sodium hypochlorite) in laboratory toxicity tests. Fish that died after exposure to lethal concentration to chlorine showed no vertebral damage (Figure 10A) nor did fish swimming in water containing sublethal concentrations. Couch believes that the evidence strongly suggests that Kepone, alone, is a sufficiently specific toxic chemical to elicit the vertebral effect. Specimens of dead fish with broken backs taken from the James River were preserved in alcohol and stored. In 1973-74 the discharge of Kepone into the James River had not been thoroughly investigated. In 1976, Couch studied in the laboratory the effect of Kepone on the sheepshead minnow and found among other observations that Kepone caused scoliosis, a lateral deviation of the spine; at high doses Kepone caused the back to break during tetanic convulsions (Couch et al. 1977). This breakage was established as a dose dependent, time dependent, predictable response. The spinal break occurred (Figure 10B) after exposure of fish to parts per billion concentrations of Kepone. Subsequently, the James River specimens that had been stored in alcohol for 3-4 years were analyzed for Kepone, and the fish muscle and preservative alcohol were found to contain parts per million concentrations of Kepone. Scoliosis has been observed in a number of the fish species as a result of parasitic infections, dietary deficiencies, exposure to heavy metal and after organochlorine, organophosphate, and carbonate poisoning (Couch et al. 1979). What killed the James River fish? Probably a combination of events and causes, with Kepone poisoning a major factor. Perhaps other factors, such

Figure 10. (A) Original X-ray of several Cyprinodon variegatus exposed to 89 µg/L chlorine (sodium hypochlorite). Exposure was lethal to fish; however X-ray shows no vertebral breaks or other abnormalities. (B) Original X-ray of a bluefish collected from the James River in 1974. Note break of vertebrae column. Concentrations of 2.8 to 4.8 mg kg^{-1} were found in bluefish tissue samples analyzed in 1977. (C) Whole longitudinal horizontal section of Cyprinodon variegatus exposed to Kepone. Fish had advanced scoliosis; note fracture in spine.

as chlorine poisoning, also acted to trigger the lethal events. The James River is polluted with many chemicals, as are many rivers and estuaries in the United States. Sorting out causes of anomalies in field specimens thus remains a challenge.

CONCLUSIONS

1. Severe pathologies occur in some marine fish populations, sometimes tentatively associated with pollution, but usually the association is tenuous and difficult to draw conclusively.
2. In almost all cases the etiologies of the lesions, including cancer, are unknown because nonspecific lesions normally occur, field specimens are collected from regions that are contaminated by many different chemicals, and the site of exposure of mobile species is often unknown.
3. The type of lesion offering the best potential diagnosis is the nonspecific inclusion body type of lesion which may contain the toxicant eliciting its formation.

RECOMMENDATIONS

If an anomaly in a field specimen is thought to be caused by a specific toxicant, it is necessary to:

1. Establish a strong correlation between pathology in the fish in the wild and degree of anthropogenic pollution.
2. A test species from the pollution region must be found that survives the highest pollutant concentration, exhibits the severest pathologies (e.g., cancer), and can be cultured in aquaria or pools.

The best test species appear to be the flatfish, which are easy to maintain in aquaria and often enter the most polluted environments where they are in direct contact with the sediment and feed on contaminated benthic organisms. Although flatfish often do not die immediately or become repelled by heavy pollution, their tissues, particularly liver, appear to be quite sensitive to pollution stress. Thus, flatfish represent an underutilized organism with significance in the field.

The approach would be to introduce the test species (caged or tagged) into a polluted region and collect and examine specimens periodically for progressive pathological changes, and to induce the pathology in laboratory tests

employing sediment or food from the polluted area. The sediment or food can be fractionated and the agent causing the lesion isolated and tested in a bioassay.

This approach has been successfully applied by Ishio et al. (1972 a, b) to isolate the cause of tumors in the commercially important marine alga Porphyra tenura in Japan. In bioassays carried out with the alga over a 38-day period, they isolated two chemical carcinogens from sea bottom muds. These carcinogens caused algal abnormalities and were subsequently traced to a coal chemical industry. Thus, although there are undoubtedly additive and synergistic factors involved in any pollution-induced lesion, there may be only a few specific toxicants that act as primary agents in the etiology of a given lesion. This study by Ishio et al. (1972 a, b) shows that the cause of tumors in a marine plant population can indeed be traced to specific chemicals in the environment of that plant, a useful model for similar studies in the future.

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